SPECIFIC DEGRADATION OF HISTONES H1 AND H3

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Summary

It is shown that acid treated histones H1 and H3 are susceptible to specific degradation by an associated acid resistant protease. Dialysis against distilled water (pH 5.5-6) of the acid treated histones enhances proteolysis. On the other hand, no degradation is observed in nucleohistone either in the presence of Ca^{++} or Na^{++} ions. The conditions required to avoid degradation during nucleohistone and histone manipulation are described.

The degradation of histones by chromatin associated proteases has been studied in different laboratories. One of these proteases shows specificity for H1 and H3 when these histones are associated to DNA, but appears to be unspecific when the histones are free in solution, so that all histones are degraded in that case (1,2). This enzyme has been purified and partially characterized by Kurecki and collaborators (3). They have shown that it is a serine type enzyme. In this paper we demonstrate that isolated histones under certain conditions are also susceptible to a specific degradation of H1 and H3, while no proteolysis of the other histones is detected. We conclude therefore that the specificity of this protease is not necessarily due to a peculiar conformation of histones H1 and H3 in nucleohistone. As a practical consequence, our experiments show that degradation

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can be avoided by operating at a low pH, an observation which should be taken into account in order to prevent histone degradation during chromatin studies.

Materials and Methods

All work was carried out at 0°-4°C. About 20 g of frozen calf thymus were homogenized in 200 ml of 0.15M NaCl, 10^{-3}M EDTA, pH=7.8 in a Waring type blender (Berrens), strained through 6 thickness of cheese-cloth and centrifuged at 2500 g for 10 min. This homogenization was repeated three more times, adding 0.1 % Triton X-100 in the second step, in order to remove lipids. The sediment was then homogenized in 500 ml of 10^{-4}M EDTA, 10^{-3}M Na Cacodylate, pH=7.8 for 15 sec. at half speed. The viscous solution obtained was transferred into a 1 liter beaker and stirred at medium speed for an hour. Very gently stirring was continued overnight and the solution was then centrifuged at 8000 g for 20 min. The clear supernatant was recovered. Its DNA content was about 1-1.4 mg/ml. The sediment was small and was discarded.

In order to determine the eventual influence on proteolysis of different treatments of nucleohistone, aliquots were treated with either 0.15M NaCl or $5x10^{-3}$M $CaCl_2$, 10^{-3}M Tris-HCl, pH=7.8. Practically all the nucleohistone precipitated in the first case, whereas a supernatant and a sediment resulted from the Ca^{++} treatment after centrifugation for 20 minutes at 3000 g. The precipitates were redisolved in 2M NaCl.

In order to isolate the histones, the DNA was precipitated from nucleohistone by adding a measured amount of concentrated HCl which made the solution 0.25N in this acid. This solution was dialyzed either against distilled water or 0.25N HCl. The histones were then recovered by precipitation with 6 volumes of acetone.

Polyacrylamide gel electrophoresis of histones was carried out according to the method of Panyim and Chalkley (4), using 6.25M urea. Best results were obtained with tubes of 8 mm diameter and 18 cm length, run during 24-25 hours at 1.25 mA/gel, 150-170 volts.

Results and Discussion

In Figs. 1 and 2 we show the histones obtained after different treatments of nucleohistone. Degradation bands appeared when nucleohistone, precipitated with either Ca^{++} or Na^{++}, was redissolved in 2M NaCl, treated with HCl and the histone solution dialyzed against distilled water

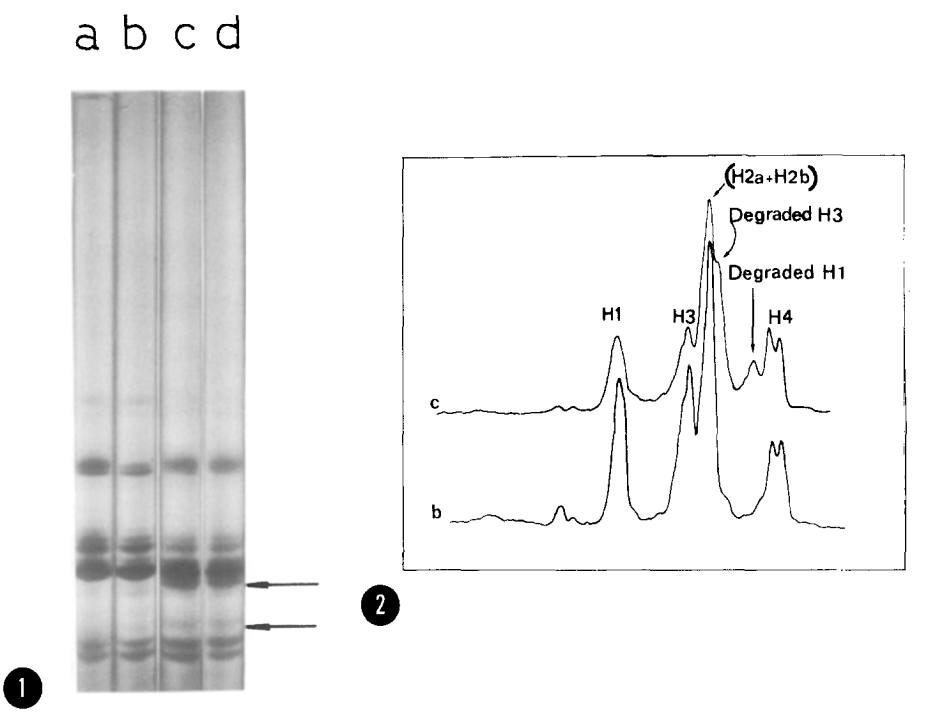

Fig. 1. Polyacrylamide gel electrophoresis of histones: (a) directly extracted with HCl, control; (b) obtained from Ca^{++} soluble nucleohistone; (c) obtained from Ca^{++} precipitated nucleohistone; (d) obtained from nucleohistone precipitated with .15M NaCl. In the two latter cases degradation bands (arrows) are obvious. In (c) and (d) the histones had been dialyzed against distilled water as described in the text. When this step was substituted by dialysis against 0.25N HCl, no degradation bands were observed and the pattern was identical to those shown in frames (a) and (b). The origin of the gels is at the top of the figure.

Fig. 2. Microdensitometer tracings of the electrophoresis runs shown in the previous figure. The degradation products are tentatively identified from the relative amount of each band. As a matter of fact degraded H3 has the same electrophoretic mobility as reported by Brandt and von Holt (6).

(pH=5.5-6.0). No degradation was observed when the dialysis of the identically treated histone solution was carried out against 0.25N HCl. It appears that histones during dialysis against distilled water are very susceptible to a residual proteolytic activity. No degradation was detected while the nucleohistone suspension was handled in the spe-

cified conditions. Neither Ca^{++} nor Na^{+} promote any degradation of the histones present in nucleohistone at pH=7.8. It is remarkable that this activity occurs after the whole proteins have been treated with acid.

Taking into account the changes observed in the quantitative amount of the different bands (measured from the microdensitometer tracings) and the results on histone degradation reported by other authors (5,6), we conclude that the main degradation product is specifically cleaved H3. Cleavage probably occurs at a specific peptide bond, as shown by Brandt and Van Holt (6). An additional broader band is observed, which we have tentatively identified as due to H1 degradation. These observations are in partial agreement with results reported by Bartley and Chalkley (1) who found that in whole nucleohistone, H1 and H3 are the two histones most easily degraded. Here, however, we show that degradation also occur in the isolated histones even after acid treatment. This fact indicates that the specific degradation of these histones is not due to a peculiar conformation occurring in nucleohistone. The biological significance of this acid-resistant proteolytic activity remains to be elucidated.

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